

Modulators of Large-Conductance Calcium-Activated Potassium (BK) Channels as Potential Therapeutic Targets

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Abstract: BK channels are large conductance calcium-activated potassium channels that are found in many tissues, including excitable cells such as myocytes and neurons. The high conductance and dependence on calcium of BK channels suggests that modulation of these channels may have a pronounced effect on tissues in which they are expressed. Within the past four years, a variety of small molecules and natural product-derived modulators of BK channels have been described. This review will focus on compounds which are openers and blockers of BK channels and their therapeutic potential, with introductory sections covering the biophysics, pharmacology and molecular biology of BK channels.

Introduction

Potassium (K^+) channels are membrane-spanning proteins which regulate cellular K^+ homeostasis and thereby play an important role in many cellular functions. In excitable cells such as nerves and muscle, among their various functions, K^+ channels shorten the duration of the action potential and, for some K^+ channels, determine the cellular resting potential. In general they repolarize the cellular membrane after excitation and reduce the effect of synaptic inputs when they are opened through native or pharmacological stimuli [1]. The fundamental modulatory function of K^+ channels and the wealth of information which has recently been obtained regarding the function and structure of K^+ channels has made them an important therapeutic target that has attracted much attention in recent years.

K^+ channels can be classified into several families based upon their primary amino acid sequence and biophysical characteristics [2]. From a pharmaceutical perspective, two types of K^+ channels have emerged as potential therapeutic targets for drug development. These are the ATP-sensitive (K_{ATP}) channel, a member of the inward rectifier family in which co-expression of a modulatory subunit confers many functionally and pharmaceutically important properties [3], and the large conductance (BK or big K, maxi-K) calcium (Ca^{2+})-activated K^+ channel, recently shown to be a single-member family (SK_1) within the voltage-dependent K^+ channel superfamily *vide infra*. These are the only K^+ channels for which relatively specific openers (activators) have been described.

Historically, K_{ATP} channels attracted the attention of numerous pharmaceutical companies, following the discovery that the mechanism by which the anti-hypertensive agent (±)-cromakalim lowered blood pressure was a result of the activation of potassium channels [4]. These efforts initiated a new era in research related to K^+ modulation, and spawned continuing efforts to identify even more effective openers. Second, third and fourth generation K_{ATP} openers have been discovered, and clinical trials are underway or planned, to treat diseases such as hypertension, cardiac arrhythmias, asthma and urinary incontinence [5,6]. The recent elucidation of the

molecular biology of K_{ATP} channels will contribute significantly in future drug development by providing well defined molecular targets for drug discovery. BK channels have only become practical drug targets in the past 4-5 years, since the discovery of the first small molecule and natural product-derived BK modulators [7,8].

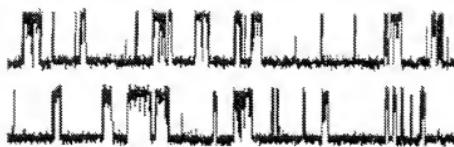
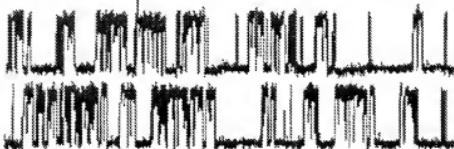
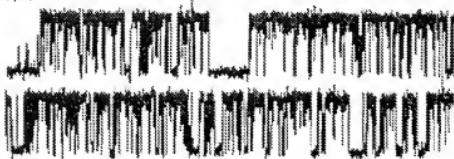
The subject of this review is the modulation of BK channels: this pharmacology is rapidly expanding. The BK channel was first cloned from the *Stopoke (ds16)* locus of *Drosophila* in 1991 [9]. The subsequent expression of the *Sk1* protein in *Xenopus* oocytes revealed that it formed a functional BK channel [10] and cloning and expression of the mouse [11] and human [12-15] *Sk1* BK channel has served to unify the research interest in this class of potassium channel. Since the initial reports of small molecule modulators, BK channels have recently been shown to be regulated by a variety of compounds from which channel openers and blockers have been identified. Several reviews have appeared which discuss the status of BK channel modulators known at the time [6,8,16]. This review examines BK channel structure and function with an emphasis on effects of both openers and blockers and their possible therapeutic utility. Because this field is relatively new, much of the information regarding different channel modulators comes from the patent literature and is therefore incomplete by virtue of the lack of information on negative results and the limited data which is disclosed.

BK Channel Biophysics and Pharmacology

Recent advances in molecular biology and biophysics have allowed for rapid progress in the characterization of the structure and function of ion channels in general, and K^+ channels in particular. The techniques of patch clamp recording from single ion channels, and the cloning of specific SK_1 channel genes, permit an unprecedented level of analysis of the biophysical characteristics of ion channels. As a consequence, the development of pharmacological tools and therapeutic agents has progressed in a previously unknown manner. Within the last several years these techniques have been applied to the analysis of BK channels, leading to new insights concerning the molecular basis of BK channel biophysics and modulation, the distribution of the distribution of these channels, and the rapid development of openers and blockers of BK channels. However, much of our understanding of the function of BK channels, especially as it

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Control

NS 004 10 μ MNS 004 50 μ M

Recovery



10 pA
0.1 s

Fig. (1). Example of BK channel activation by the substituted benzimidazolone BK opener NS 004. The recording was obtained from a native neuronal BK channel excised from a hippocampal neuron in culture, and recorded in the inside-out patch-clamp configuration. The estimated free Ca^{2+} concentration on the extracellular side was 1.0 μM , and the K^+ concentration was symmetrical at 150 mM. The drug's effects had a rapid onset; note that the effect of NS 004 was quite significant and concentration-dependent, and was completely reversible after a brief period of recovery in the control solution.

relates to therapeutic utility, continues to come from recordings obtained from both native and cloned channels.

While BK channels share many biophysical characteristics with other voltage-gated K^+ channels, they possess many features that are unique and which lend themselves to the specific targeting of these channels for drug development. The BK channels are one of two major classes of Ca^{2+} -gated K^+ channels, the other class consisting of the largely voltage-independent SK (small K^+) channels. A typical single channel recording in the absence and

presence of a BK channel opener is shown in Fig. (1). A third class of Ca^{2+} -dependent K^+ channel, 'intermediate K^+ ', may also exist. The identity of BK channels as a separate class of voltage-dependent K^+ channels has now been confirmed with the recent cloning of a single member of a unique gene family, *Slc9a6*.

Native BK channels have been shown to exist in a number of different phenotypic variants. Although no comprehensive classification of the c channels has yet been defined, one system has attempted to group the channels encountered in brain into two

classes (Type I and Type II) based on their steady-state kinetics, modulation by protein phosphorylation, and sensitivity to the peptide toxin blocker charybdotoxin (CnTX) [17-20]. While BK channels are phenotypically quite diverse, they all share certain important features that make them functionally important for cell regulation, and suggest that they would be favorable targets for therapeutic development. Most prominently, they are dependent upon both voltage and intracellular Ca^{2+} [21-24], and have very large single-channel conductance values, typically far in excess of 100 pS in symmetrical K^+ solution [17,24-28]. They activate rapidly, and with rare exception, they are non-inactivating or only slowly inactivating [28-32]. In addition to activation by voltage and Ca^{2+} , many other factors influence the activity of BK channels and may represent physiological mechanisms of channel modulation. Prominent among these is membrane phosphorylation, while the consequences of BK channel phosphorylation are still incompletely characterized, it is clear that several second messenger systems can participate via activation of different kinases, and that phosphorylation can produce an increase or a decrease in channel activation, perhaps depending on the kinase and the BK channel phenotype [18,19,33,34].

The degree of Ca^{2+} sensitivity for particular native BK channels is variable, with muscle BK channels generally being somewhat more sensitive to intracellular Ca^{2+} than neuronal channels [22-24]. At physiological intracellular Ca^{2+} concentrations, which are quite low at rest, the strong voltage-dependence of BK channels guarantees that there is little channel activation at, or near, resting membrane potentials. During and following cellular activation, membrane depolarization and voltage-dependent Ca^{2+} entry both contribute to activation of BK channels, resulting in membrane repolarization and a block of further Ca^{2+} entry. One phenomenon that has been documented in several systems is the 'twisting' of voltage-dependent Ca^{2+} channels and BK channels [35-39]. This refers to the co-localization of these channels in regions where close regulation of the concentration of intracellular Ca^{2+} is critical, such as in presynaptic boutons of neurons [36,37]. This may be particularly important and prevalent in neurons, as BK channels in these cells are generally less sensitive to Ca^{2+} than their counterparts in other tissues, and short diffusion distances would be required for tight temporal control of Ca^{2+} entry and the efficient regulation of transmitter release. The mechanism of this channel co-localization is not currently understood. Because of their relatively fast activation kinetics and low level of inactivation, most BK channels are able to quickly respond to changes in both membrane voltage and intracellular Ca^{2+} , and remain at an enhanced level of activation until resting conditions are restored.

Until quite recently, information concerning the localization of BK channels came from physiological experiments where the identification of BK channels in particular tissues relied on isolating from identified channels or by testing the effects of pharmacological agents on specific functional responses. The most useful tools in this regard have been the peptide toxins CnTX and particularly inactivation (hTx), which is very specific for BK channels [40-42]. These studies have shown that BK channels are localized in many types of excitable and non-excitable tissues [27,42-51]. With the cloning of BK channels (*vide infra*) more detailed analyses of BK channel localization have begun. Recently a comprehensive examination of the distribution of these channels in brain tissue, confirmed that BK channels are found in many important regions of the brain, prominent among these are the hippocampus, cortex, and various subcortical nuclei. It has also been

found that the channels are preferentially transported to, and inserted in, the presynaptic bouton [51]. These data taken together indicate that BK channels are present in a number of organ systems, and are likely very important in the control of neuromodulation and hormone release.

Molecular Biology of BK Channels

It is well known that ion channel proteins are involved in generation, propagation, and integration of electrical signals in excitable cells, as well as regulation of numerous other functions in virtually all cells. Various types of K^+ currents from different tissues and isolated cells have been dissected, suggesting the existence of a large molecular family of K^+ channels. The concept of diversity of K^+ channels was validated with the identification of specific genes mediating different ion currents. The first molecular insight into understanding K^+ channel proteins and the familial relationships among channels occurred with the cloning of a family of K^+ channel genes from the *Shaker* locus of *Drosophila* [54]. Since the initial report of the *Shaker* gene, a large number of novel voltage-gated K^+ (K_v) channel cDNAs have been cloned, for both vertebrates and invertebrates, that are responsible for action potential repolarization in excitable cells and regulation of secretion in gland cells.

The molecular biological breakthrough for BK channels followed the same path as for the *Shaker* family. Through the use of molecular genetics, a flight muscle mutation known as 'slipwedge' was determined to lack a Ca^{2+} -activated K^+ current, leading to an inability to repolarize the muscle following the action potential [55]. This discovery enabled the isolation of a gene, termed *Slc*, encoding a BK channel [59]. Confirmation that the *Slc* gene, in fact, formed a functional BK channel came with the complete cloning of the 5' end and expression in *Xenopus* oocytes [56]. Adelman and colleagues were able to show that the resultant *Slc* (*Drosophila*-*Slc*) channels had single channel conductance in equimolar K^+ of 126 pS and channel opening was dependent on both the extracellular free Ca^{2+} concentration and membrane potential. One striking feature of *Slc* was the presence of five alternative splice sites with multiple alternative exons per site which allowed for a large number of channel variations. It was subsequently shown that functional differences in unitary conductance, calcium sensitivity, and activation kinetics resulted from the insertion of different alternative *Slc* splice exons [53]. These data support the proposal of wide phenotypic diversity from the expression of one gene.

Subsequent to the identification and isolation of *Slc*, low stringency hybridization screening of transhuman cDNA libraries resulted in first, a mouse homologue (*mSlc* [11]), followed by the cloning of a human BK channel, *hSlc* [12-15]. Functional expression of cloned *hSlc* channels revealed a high degree of voltage- and Ca^{2+} -dependence, with an average single channel conductance of 286 pS, which is similar to native channels [12].

The structure of the *hSlc* BK channels, in part, is very similar to the voltage-gated potassium channel superfamily with an putative membrane spanning domain and highly conserved sequences within the pore and voltage sensor domain. However, these BK channels also contain a long carboxyl terminus with four additional hydrophobic segments. The membrane topology has been computationally generated based on the hydrophobic regions of the translated DNA sequence, but evidence is lacking that describes the role of each hydrophobic domain. Although computer derived hydrophobic plots may indicate the presence of membrane spanning domains, it

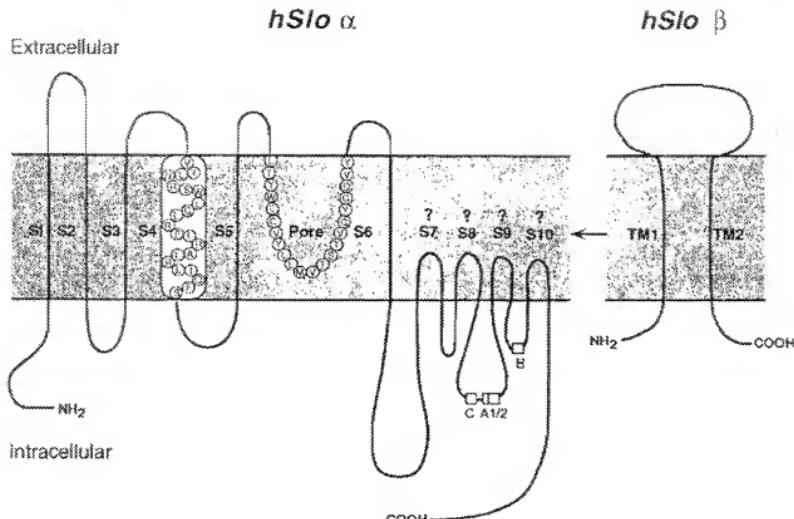


Fig. (2). Schematic representation of *hSlo* α and *hSlo* β . The *hSlo* α protein is depicted with 6 membrane spanning domains, S1-S6, a pore domain, and 4 additional hydrophobic domains labeled S7-S10. The topography of S7, S8, S9 and S10 remains unknown. One possible scenario is the looping of these hydrophobic segments into and out of the membrane. The amino acids are illustrated in the pore and S4 regions to highlight the highly conserved sequence characteristics of potassium channels; the TVGYG in the pore and the R-R-R repeat in the S4 voltage sensor. The *hSlo* β has two putative transmembrane domains labeled TM1 and TM2. The *hSlo* β protein interaction with *hSlo* α remains to be determined.

remains unclear whether these four additional segments, S7-S10, of BK channels cross the membrane, extend into the membrane or are entirely cytoplasmic. Fig. (2) shows one possible topology of the α subunit (*hSlo*) and recently cloned human β subunit. The figure shows the S1-S6 membrane spanning domains passing through the membrane, the pore within the membrane, and the four additional hydrophobic domains dipping into the membrane from the intracellular surface. The location of the three identified human splice sites are indicated by the small boxes marked A, B, and C between the S7-S8 and S8-S9 linkers. The amino acids within the S4 and pore regions are depicted to show the two most distinguishing characteristics of voltage-gated potassium channels; the GYG pore sequence and the R-R-R sequence within the S4 voltage sensor.

A major ongoing effort in the molecular biology of BK channels is directed at probing the contribution of single amino acids or domains to specific BK channel properties as well as determining the stoichiometry of the channel. The use of site-directed mutagenesis of the critical tyrosine residue within the vestibule of the *dSlo* pore, which is responsible for the high affinity binding of TEA, provided evidence for the stoichiometry of the functional channel. Expression of a mixture of wild type channel and the point mutant channel revealed four discrete amplitudes with application of TEA, suggesting that the functional BK channel, like three other K $^{+}$ channels, is a tetramer [56]. The precise location of

the Ca $^{2+}$ sensor remains unknown. However, by taking advantage of differences in Ca $^{2+}$ sensitivity between *mSlo* and *dSlo*, Wen and colleagues found that the 'core' and 'tail' domains were expressed individually; the tail region of *mSlo* conferred the increased *mSlo* Ca $^{2+}$ sensitivity on the normally less sensitive *dSlo* core channel [57]. The corresponding experiment also showed that the *dSlo* tail conferred *dSlo*-like low Ca $^{2+}$ sensitivity on the *mSlo* channel. This suggests that the Ca $^{2+}$ sensor region is in the carboxy terminus of the *Slo* protein.

The voltage-gated potassium channel superfamily has four known subfamilies, with multiple genes within each family, leading to a tremendous diversity of these channels. The identification of several β subunits that alter rates of inactivation further increases the diversity of the voltage-dependent K $^{+}$ channels [50,51]. Although the phenotypic diversity of BK channels is well documented by numerous electrophysiology studies from different tissues and cell types, to date only one gene encoding for BK channels has been identified. Low stringency homology screening and PCR experiments have not been able to identify new BK gene family members. Similar to the voltage-gated family, a β -subunit that interacts with the BK α -subunit was identified by biochemical purification from bovine smooth muscle [58]. Cloning of the bovine β -subunit revealed that it does not form a channel by itself, but co-expression of the β -subunit with the *mSlo* α -subunit showed an increase in calcium sensitivity and responsiveness to the BK

channel opener DHS-1 [24]. A further analysis of this interaction using human α - and β -subunits indicated several phenotypic alterations of the *hBK* channel by *hSlo* β -subunit co-expression [59]. Changes in blocker sensitivity, activation, relaxation and inactivation kinetics and PKA-modulation were also observed. The BK β -inhibitory may therefore play an even more important role in altering BK channel characteristics than β -subunits for voltage-dependent K^+ channels.

Overall, the combination of molecular biology and electrophysiology has played a seminal role in our understanding of ion channel function, which in turn has aided in the screening of new chemical entities that modulate ion channel function. The cloning of a human gene, and expression in a relatively isolated background, has enabled the screening of new synthetic compounds which will further delineate emerging structure-activity relationships among the BK channel modulators.

BK Channel Openers

Small Molecules

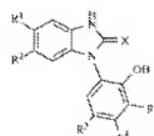
Benzimidazolones

NeuroSearch first introduced the concept of small molecule activators of BK channels in 1992, as disclosed in a series of N-aryl benzimidazolones. Typified by NS 004 (1), and later by NS 1619 (4, Table 1) [8,60], these patents described the activity of the benzimidazolones in several types of experiments using the patch-clamp technique. The data from the experiments are typically reported over a range in which the compounds activated BK channels. As measured in inside-out patches from cerebellar granule cells, the active compounds reported contained

Table 1. Benzimidazolone BK Activators

benzimidazolones substituted at the N-1 position and with a 2-phenyl moiety. Phenols with electron withdrawing groups (NS 004 and NS 1619) activated BK channels at concentrations of 3-10 μ M and 3 μ M, respectively, and were slightly more active than bis(2-phenyl)phenol 3, and much more active than unsubstituted phenol 2. A similar trend was evident for benzimidazolones evaluated in cultured bovine aortic smooth muscle cells, where again all of the activators reported contained electron-deficient benzimidazolone rings with N-substituted aryl groups. The most active analog in this series, compound 8 (1 μ M), was also the most electron deficient on both the benzimidazolone and phenyl rings. One example of a thienobenzimidazolone was reported (9) and stands out as the only active compound reported in either assay which contains an unsubstituted phenolic residue (3-30 μ M).

Because the benzimidazolone BK openers are small molecules and synthetically accessible, they have been studied in a diverse array of cell types using a variety of electrophysiological techniques. The activity of NS 004 has been characterized using BK channels found in mouse cerebellar granule cells [61], cultured calf aortic smooth muscle cells [62], rat GH3 clonal pituitary tumor cells, channels from rat cortex reconstituted into planar lipid bilayers, and cloned *Drosophila Slo* BK channels expressed in *Xenopus* oocytes [25]. NS 004 effectively opened BK channels from these cells and, perhaps more importantly, channel opening occurred with both the extracellular and intracellular application of compound. The net effect of NS 004 was to shift the activation curve towards a more negative membrane potential. In pulmonary artery smooth muscle cells, the activation of BK channels by NS 004 can be regulated by varying the concentration of free calcium; elevating the intracellular calcium levels induces a potentiation of NS 004-induced activation [23]. This suggests a synergism between Ca^{2+} and NS 004 in the activation of BK channels.



Compound	R ¹	R ²	R ³	R ⁴	R ⁵	X	Cerebellar Granule Cells (μ M)	Activation of Mouse Aortic Smooth Muscle Cells (μ M)
1 (NS 004)	CF ₃	H	O	H	H	O	3-10	3-30
2	CF ₃	H	H	H	H	O	40,000-50,000	—
3	CF ₃	H	Ph	H	H	O	3-30	—
4 (NS-1619)	CF ₃	H	CF ₃	H	H	O	3	—
5	Cl	Cl	Cl	H	H	O	—	4-10
6	CF ₃	H	CH=CH-CH=CH	H	H	O	—	3-30
7	CF ₃	H	Ph	H	H	O	—	3-30
8	CF ₃	NO ₂	Ph	H	NO ₂	O	—	1-3
9	CF ₃	H	H	H	H	S	—	4-10

At concentrations of $\leq 30 \mu\text{M}$, both NS 004 and NS 1619 induced concentration-dependent increases in the open state probability of BK channels present in bovine tracheal smooth muscle [65]. When examined at higher concentrations in oocytes expressing *hBK*, both compounds produced a maximum current in excess of 300% of control values [64]. Calculation of the absolute maximal effects and accurate estimates of IC_{50} values were prevented by limited solubility at concentrations greater than $100 \mu\text{M}$; limited solubility in aqueous buffers is a recurring problem with these lipophilic compounds. NS 1619 also activated BK channels from a variety of different tissues, including smooth muscle cells from bovine aorta and coronary artery, mouse cerebellar granule and cortical cells, rat pancreatic β cells [28], and in BK channels in membrane patches isolated from rat ventromedial hypothalamic neurons [53]. Examining single channel recordings of BK channels from neurons isolated from rat motor cortex, NS 1619 induced concentration-dependent activation of channels with a calculated EC_{50} of $32 \mu\text{M}$ [30].

In addition to activating BK channels, NS 004 exhibits activity in other types of ion channels. The effect on the channels has, in some cases, been dependent upon the tissue source of the channel. For instance, NS 004 activates K_{ATP} channels isolated from guinea pig ventricular cells, however, it inhibits K_{ATP} channels found in canine coronary artery [65]. Similar tissue-selective activity has been observed with NS 1619; it does not modulate K_{ATP} channels present in rat ventromedial hypothalamic neurons [53] or rat pancreatic β cells [66], however, it has been reported to inhibit

K_{ATP} channels in smooth muscle cells isolated from rat intact portal veins [67]. It also has been reported to block both K_v channels and L-type calcium channels in rat intact portal veins [67], and K_v and Ca^{2+} channels from other natural sources, as well as cloned K_v channels [65,68,69]. However, no measurable effects were induced by $10 \mu\text{M}$ NS 1619 in K_v channels from either mouse cerebellar granule cells or mouse cortical neurons or in calcium channels obtained from mouse cerebellar granule cells [66]. Finally, NS 004 activates the CFTR Cl⁻ channel, including at least two mutant forms, [70,71] and increases Ca^{2+} -activated Cl⁻ current in *Xenopus* oocytes [27].

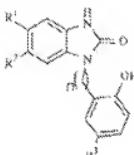
As an extension of the benzimidazolines, a group of structurally related benzyl benzimidazolones have been prepared in an effort to examine some of the fundamental structure-function relationships in these series [72]. Table 2 shows the BK activity as a percent of hBTX-sensitive current using two electrode voltage clamp recordings from *Xenopus* oocytes expressing the cloned BK channels *mSh* or *hSh*, the mouse and human *Sh* homologs, respectively. Insertion of a methylene spacer between the benzimidazolone and chlorophenol nucleus of NS 004 provided methyl alcohol 10, which demonstrated comparable activity to NS 004. Removal of the trifluoromethyl group on the benzimidazolone or replacement with a methyl group leads to loss of activity (13 and 14, respectively). Unlike the NS 004 series, in which removal of the chlorine resulted in a thousand fold decrease in activity (2, Table 1), removal of the chlorine of 10 to give 12 resulted in only a slight diminution of activity. Replacement of the 5-trifluoromethyl group of 10 with halogens or nitro either retained or enhanced channel opening (15-17). Moving the chlorine (15) or bromine (16) from the 5- to the 6-position (18, 19) resulted in a decrease or loss of activity, respectively.

An acyclic form of the benzimidazolones has been reported in the form of diaryl ureas (Table 3) [73]. When examined for BK activation in cultured bovine rectore smooth muscle cells, NS 1608 (26), the acyclic form of NS 004, was the most active compound disclosed. Interestingly, 2-hydroxy-5-trifluoromethyl urea 22, the acyclic form of NS 1619, only activated BK channels at concentrations greater than or equal to $10 \mu\text{M}$. This is in contrast to the similarity in activity between NS 004 and NS 1619 in a variety of different assay systems as described above. The effects of NS 1608 have also been examined on whole cell current in porcine atrial cells using the patch-clamp technique [74]. When employing step depolarizing pulses from a holding potential of 0 mV , NS 1608 produced an increase in outward current compared to control after administration at a concentration of $5 \mu\text{M}$. Paradoxically, when administered at a concentration of $50 \mu\text{M}$ under the same conditions, a reduction in current as compared to control was observed. It is speculated that the variable effect of NS 1608 is a result of dual mechanisms of action which are, as yet unidentified, but may simply reflect secondary pharmacological blockade of non-BK components of the outward current. It is also possible that the reduction in current is a result of decreased selectivity, resulting in a lack of activity at the higher concentrations.

Biaryl Amines

Another group of small molecule BK openers is represented by the farnesol, arachis, stearic, linoleic acids (Table 4). Farnesol and Palmitoleic acids are known to possess neuroinflammatory and anti-inflammatory activity and to inhibit c-fms-like kinase in both a *Xenopus* oocyte expression system [75] as well as chloride

Table 2. Benzyl Benzimidazolone BK Activators



Compound	R ¹	R ²	R ³	n	Outward Current as % of Control Current ^a
1 (NS-004)	CH ₃	H	Cl	6	192 \pm 13
10	CH ₃	H	Cl	1	133 \pm 9
11	CH ₃	H	H	2	119 \pm 12
12	CH ₃	H	H	1	126 \pm 10
13	H	H	Cl	1	113 \pm 13
14	CH ₃	H	Cl	1	102 \pm 11
15	Cl	H	Cl	1	180 \pm 16
16	Br	H	Cl	1	136 \pm 8
17	NO ₂	H	Cl	1	163 \pm 10
18	H	Cl	Cl	1	110 \pm 10
19	Cl	Br	Cl	1	108 \pm 7

^a Compounds were evaluated at a concentration of $30 \mu\text{M}$; a value of 100% indicates no effect.

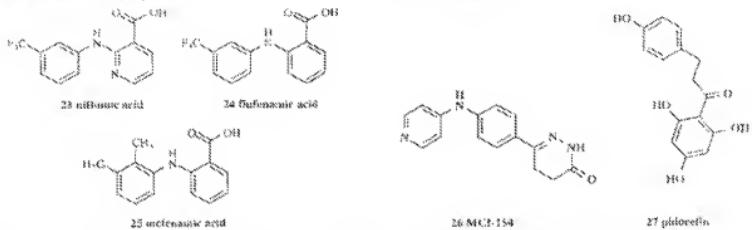
Table 3. Bicyclic BK Openers

Compound	R ¹	R ²	Activation of Cultured Bovine Aortic smooth muscle cells (μM) ^a
20 (FS-1698)	CF ₃	Cl	1
21	H	Cl	≥3
22	CF ₃	CF ₃	≥10

^aConcentrations where significantly activated the BK channel.

channels obtained from cultured monolayers of dog and bovine aortae [76]. These compounds have also been found to open BK channels present in coronary smooth muscle cells reconstituted into lipid bilayers [77]. When iniflumic acid was applied to the external side, it opened BK channels by left-shifting both the voltage- and calcium-activation curves. A direct comparison of the three acids in this system showed that iniflumic acid and ibufenamic acid were nearly equivalent in activating BK channels, however, mefenamic acid was a less effective activator. Interestingly, all compounds were more active when applied to the external side of the membrane.

Table 4. Fumamic Acid BK Openers



Compound	Normalized Increase in BK Channel Activity ^a	I _{Drug} /I _{Control} @ 80 mV (nA) ^b
	External ^c	Internal ^c
23 (iniflumic acid)	0.78	0.06
24 (ibufenamic acid)	0.77	0.17
25 (mefenamic acid)	0.69	0.03
FS-984	1	1

^aExternal application of 100 μM drug.

^bInternal application of 100 μM drug.

^cComparison of relative BK current normalized to control open current at 100 μM drug.

Another direct comparison of the three acids was conducted using BK channels present in canine coronary smooth muscle cells

measuring whole-cell current using the suction pipette method [78]. This study also demonstrated the greater effectiveness of iniflumic and ibufenamic acid over mefenamic acid in activating BK channels. In addition, a comparison of the activity of the fumamic acids was made with the activity of NS-004 at a holding potential of 80 mV and a concentration of 100 μM. NS-004 exhibited much greater BK opening activity than any of the acids (Table 5). A study of iniflumic acid and ibufenamic acid in *hSlo* expressed in *Xenopus* oocytes revealed that both iniflumic and ibufenamic acids were effective in increasing BK-mediated currents [64]. A direct comparison with NS-1619 in excised patches from healthy and transiently transfected HEK 293 cells expressing *hSlo* revealed that iniflumic acid was less effective than the benzodioxoleone in producing a leftward-shift in the half-activation voltage of these channels when applied to the cytosolic surface of the membrane. In a recent communication which studied the effects of NS-1619 and iniflumic acid in single channel recordings of BK channels made from rat minor cortex neurons, iniflumic acid had no direct effect on BK activity, however, at a concentration of 100 μM, it significantly inhibited the activating effect of 40 μM NS-1619 [79]. It was suggested that the two compounds may interact with the same sites at the BK channels and furthermore induce differential effects upon specific members of the BK channel family.

The pyridyl amine MCI-154 (Fig. (3), 26) has recently been reported to have BK channel activity [80]. In cell-attached patches obtained from porcine coronary artery smooth muscle cells, no activity was observed when MCI-154 was extracellularly applied at a concentration of 10 μM. However when the concentration was increased to 100 μM, a significant increase in BK channel open probability was seen. Similar concentration-response results were obtained when MCI-154 was applied to the cytosolic side of cell-free inside-out patches.

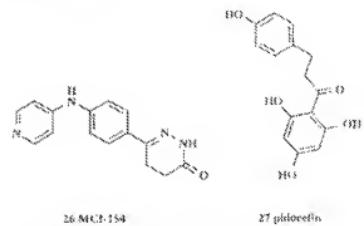


Fig. (3). The BK channel openers MCI-154 and phloretin.

Phloretin

The flavonoid phloretin (Fig. (3), 27) was studied using outside-out patches of BK channels isolated from myelinated food nerve fibers. At concentrations from 10-200 μM BK channel activity was greatly increased due to a shift of the membrane potential for half-maximal activation [81]. Application of phloretin in oocytes expressing *hSlo* resulted in significant increases in outward current. The profile of the concentration-response relationship was different from that obtained with the benzodioxoleones [64]. In particular, the data were best fit assuming two sites of interaction, with an estimated EC₅₀ of 334 μM for the high affinity site. The values for the low affinity site could not be estimated due to the limited solubility above 200 μM. In this same study it was shown that phloretin

produced significant activation of *hSlo* BK channels expressed in HEK 293 cells when applied at 25 μ M to the cytosolic surface of excised inside-out patches.

KATP Openers (Benzopyrans)

Because of their ability to act as smooth muscle relaxants, from a pharmaceutical perspective, openers of the KATP channels represent the most intensively studied group of potassium channel regulators. The interest in KATP modulation has sparked research on the effects of KATP openers on BK channels. Similar to some of the findings when BK openers were applied to KATP channels, KATP openers also have variable effects on BK channels. This may be due, in part, to the source of tissue utilized to obtain the channel. The KATP-opening benzopyran (3)-cromakalim (Fig. (4), 28) was applied to BK channels obtained from rabbit aorta and incorporated in planar lipid bilayers. The open probability of the channels increased by 56% and 260% in the presence of 50 and 500 nM cromakalim, respectively [82]. In cell-attached and inside-out patches from canine colon muscle at concentrations of 20 μ M, both the single enantiomer hemikalim (29) and also an activator of KATP channels, and the racemate cromakalim, increased the open probability of BK channels [49]. The time course of activation was much slower in cell-attached patches, suggesting that these compounds may have to diffuse through the lipid membrane bilayer in order to exert their effects. Cromakalim was also found to reversibly increase the open probability of BK channels obtained from single porcine coronary arterial cells [83]. In contrast to these studies, cromakalim failed to produce any significant effect on BK-mediated outward current in oocytes expressing BK channels [64] and in patch-clamp studies of BK channels in calf aorta [66]. This suggests that the actions of these compounds may either be indirect, or require modification of the BK channel α subunit as employed in the former study.

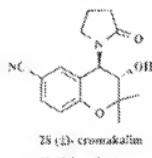


Fig. (4). Benzopyran BK channel openers.

Dihydropyridines

There is conflicting information regarding the BK channel activity of the dihydropyridines, a class of compounds extensively studied for their ability to inhibit L-type calcium channels. A study of the activity of 10 μ M nitrendipine (Fig. (5), 30) in BK channels recorded in inside-out membrane patches excised from pancreatic β -cells showed an increase in activity to 120% of control, whereas the related analog nifedipine (31) produced no effect on these channels when applied at the same concentration [84]. In contrast, when examined in outside-out patches from rat cerebellar granule cells, 1 μ M nifedipine did not affect the P_{open} or unitary conductance, however it did reduce the subconductance states of the BK channels [85]. A recent patent application by the Bayer group reported that dihydropyridine pyridine 32, an oxidized and cyclized form of the dihydropyridines, is a modulator of BK channels [86]. Specific details of BK channel activity were not provided.

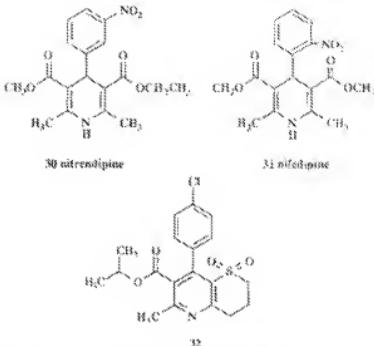


Fig. (5). Dihydropyridine-derived BK channel modulators.

Structure-Activity Relationships of Small Molecule BK Openers

One common theme that is seen in almost all of the small molecule BK openers reported to date is the concept of an electron-deficient aromatic ring linked to another aromatic ring via a nitrogen atom. Within or near the linker is an array of atoms which contain some type of hydrogen-bonding capacity. In the case of the farnamates, this is represented by a carboxylic acid and a secondary amine. The hydrogen-bonding capacity of the benzimidazolines (NS 004), the ureas (NS 1608), the farnamates (acid, nifumic acid) and the benzopyrans (cromakalim) is achieved with an enamine separated by three or four bonds from an alcohol or an amine as shown in Fig. (6). The notion that a carbonyl or

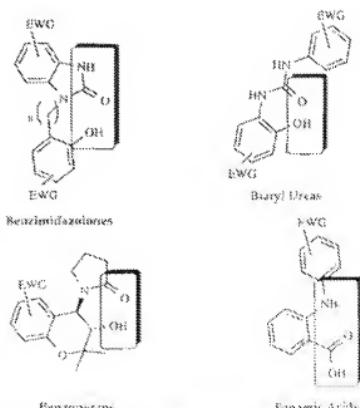


Fig. (6). Common elements among small molecule BK channel modulators.

alcohol armament in this inhibitor will interact similarly to a carboxylic acid or will not proceed; when examining structural functions of tyrosine kinase inhibitors, Kubo and co-workers noted that the ketone-phenoxy of the flavone quercetin could mimic the carboxylic acid of L-GOPA [87]. The carboxyl-alcohol may also play a role in transport of the compounds across biological membranes.

The enhanced activity observed with electron-poor systems is clearly demonstrated in the benzyl-linked benzimidazole series. Substitution of a hydrogen at the 5-position (15, 16) results in greater BK activity when compared to the corresponding 6-substituted halogens (18, 19, Table 2). An electron withdrawing group such as a chlorine or bromine *para* to the N-H, as with 18 and 19 should acidify the N-H relative to a chlorine or bromine in the *meta* position (15, 16). Therefore, the acidity of the benzimidazole N-H may be an important feature for BK activity. The necessity of an electron-poor benzimidazole to maximize BK activity is reinforced by the lack of activity when the trifluoromethyl group of 10 is replaced with the isobutene, but electron donating, methyl group (14). Removal of the chlorine group on NS 604 to give phenol 2 results in a 1000 fold decrease in BK activity (See Table 1). When the electron density of both the phenol and benzimidazole rings of NS 604 are decreased even further, such as with the addition of nitro groups in analog 8, the activity appears to also increase. A similar trend is observed with the benzyl-linked benzimidazoles 18-19, with the exception of benzyl isobutol 12. The increase of activity with this homologated analog as compared to the directly linked phenol (2), may be a result of a greater flexibility with phenol 12 which permits the adoption of a favorably interacting conformation.

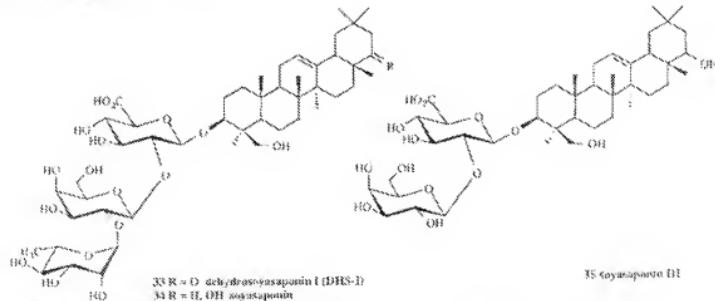
Within the heterocyclic acid series, the acidity of the NH plays a role in the BR activity. The electron-poor thiophanic and furfumic acids exhibit greater activity in several different systems than the

concomitant electric rich mefenamic acid. X-ray crystallography analysis of the isomeric acids reveal a common feature wherein the *o*-substituted ring bearing the carboxyl group is coplanar with the carbonyl and amino group by virtue of an antiperiplanar hydrogen bond between the carbonyl and the amino H-4 [88]. The interplanar angle between the pyridyl and phenyl group was determined to be 8.7° for ibuprofen acid, while the antiperiplanar angle between the two phenyl groups is 62.49° and 57.89° for mefenamic and flufenamic acid, respectively. This indicates that in the solid state, the conformation of flufenamic and mefenamic acid are similar, with the phenyl group rotated out of plane with respect to the anhydroic acid to avoid unfavorable repulsive steric contacts (H and CH_3) in the *ortho* positions of the two rings. Because ibuprofen and contains a nitrogen in the *ortho* position, it may adopt a coplanar arrangement between the two aromatic rings. This suggests that the decrease in activity of mefenamic acid with respect to ibuprofen and flufenamic acid is not due to the orientation of the aromatic rings. The difference may be accounted for on the basis of steric factors: both flufenamic and ibuprofen acid contain an electron withdrawing trifluoromethyl group on the phenyl ring, whereas mefenamic acid contains two electron donating methyl groups. With respect to the methyl substitution, the trifluoromethyl groups serve to increase the acidity of the N-H and thereby favor inter- and intramolecular hydrogen bonding. In addition, it is also possible that the *ortho* methyl group of mefenamic acid produces an unfavorable steric interaction with the receptor.

Terpenoids

The BK blocking peptide CNTX has been crucial to the process of identifying other BK channel modulators. Using a screen of compounds which inhibit the binding of ^{125}I -CNTX, several different series of terpenoid based BK activators as well as blockers

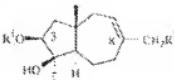
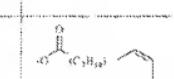
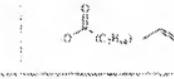
Table 5. Coherence and Binding to BK Channels



Compound	IC ₅₀ (nM)	ChTx Displacement (Ki; nM) ^a
33 (DHS-1)	2	0.1
34 (oxazepin)	2	6
35 (oxazepine-1H)	2	1

Scattered small patches of *Calluna vulgaris* and *Empetrum nigrum* are also present.

Table 5. Binding and BK Channel activity of Sesquiterpenes

Compound	R ¹	R ²	Inhibition of [¹²⁵ I]-CPTX Binding ^a	Effect on BK Channel Open Probability (10 μ M) ^b
37 (CAF-603)	H	H	IC ₅₀ \approx 200 nM	No Effect
38 (L-719,304)	H		IC ₅₀ \approx 360 nM	Increase
39		H	NE ^c	Small Decrease
40		H	31% @ 100 μ M	Small Decrease
41	H	OH	NE ^c	No Effect

^a Inhibition of [¹²⁵I]-CPTX binding with bovine sarcoplasmic membrane sources^b No effect in highest concentration tested (100 μ M)^c Interval application to excised inside-out membrane patches

have been reported by a group of Merck. The first series of openers to be disclosed was a series of triterpenoid soyasaponins isolated from a medicinal herb found in Ghana and apparently used as a folk medicine for the treatment of asthma and other diseases associated with smooth muscle contraction [17]. Table 5 shows the ability of several of the soyasaponins to displace [¹²⁵I]-CPTX incubated with bovine cardiac sarcoplasmic membranes. The most potent of these compounds, dehydrosoyasaponin I (DHS-I, 33) exhibited an inhibition constant (K_i) of 6.1 μ M. DHS-I was also shown to be only a partial inhibitor of [¹²⁵I]-CPTX binding, reaching a maximum level of 62%. Reduction of the ketone to the alcohol (soyasaponin I, 34) decreased the K_i to 6 μ M, and cleavage of the terminal sugar from soyasaponin I to give soyasaponin III (35) somewhat reduced binding (K_i = 1 μ M). Soyasaponin B, the aglycone of soyasaponin, demonstrated a K_i of greater than 100 μ M, suggesting that both the triterpene and sugar moieties are necessary for BK channel binding. When examined in cloned BK channels derived from *oocytes* *in vitro* (vide supra), DHS-I had no effect; however, when the β -subunit was co-expressed with the α -subunit, DHS-I became an *open* channel opener [21]. DHS-I was studied further in electrophysiological experiments, measuring the open probability of single BK channels from bovine tracheal smooth muscle incorporated into planar lipid bilayers. Interestingly, nothing was observed when DHS-I was applied to the outside face of the channel (500 nM), but application of 100 nM DHS-I to the inside face produced a significant increase in open channel probability. Because these compounds do not activate BK channels (consequently, it remains to be determined if the soyasaponins described here are the constituents of the medicinal herb which are

responsible for the smooth muscle relaxing properties; the ability to assess the site of action for the opener would be a requirement for drug activity.

Utilizing the same binding screen described above, a terpenoid fraction derived from an unidentified endomycete revealed the presence of a substance which bound to BK channels. Subsequent purification and structure elucidation revealed that the active component was masticidin (Fig. 7) [36] (39), which inhibited binding of [¹²⁵I]-CPTX to BK channels in bovine sarcoplasm with a K_i of 160 nM, causing a maximal inhibition of 90%. Masticidin also demonstrated an ability to activate BK channels when added to the cytoplasmic surface of excised inside-out membrane patches from bovine aortic smooth muscle cells.

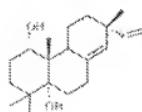


Fig. 7. The BK channel opener masticidin

A third group of terpenes to be disclosed as BK activators was identified from natural product screens of fungal fermentations extracts and is based upon the framework of CAF-603 (37). Table 6 (1996/21). Table 6 shows the inhibition of binding of [¹²⁵I]-CPTX to bovine cardiac sarcoplasm by these compounds and their effect

on BK channels in excised inside-out membrane patches measured in patch clamp experiments [91]. CAF-603 is the most potent of these analogs to block ^{125}I -CnTX binding, with an IC₅₀ of 270 nM and a maximal inhibition of 90%. Introduction of a C-17 ester tail in the 8-position of CAF-603 to give L-735,334 (38) decreased binding slightly to 360 nM, and introduction of an alcohol at the 8-position (41) completely eliminated binding. These results indicate a lack of tolerance for polar substituents at this position. Blocking the 3-OH group of CAF-603 also greatly reduced or eliminated binding (compounds 40 and 39, respectively). Interestingly, despite the ability to displace ^{125}I -CnTX, CAF-603 had no observable effect on channel open probability when applied to the intracellular side of the channel at a concentration of 10 μM . In contrast, L-735,334 caused clear, reversible increases in channel open probability when applied to the intracellular side of the channel at 10 μM . When examined in BK channels incorporated into planar lipid bilayers, application of 10 μM L-735,334 to the outside face of the channel had no significant effect on channel open probability. In excised inside-out patches at a concentration of 10

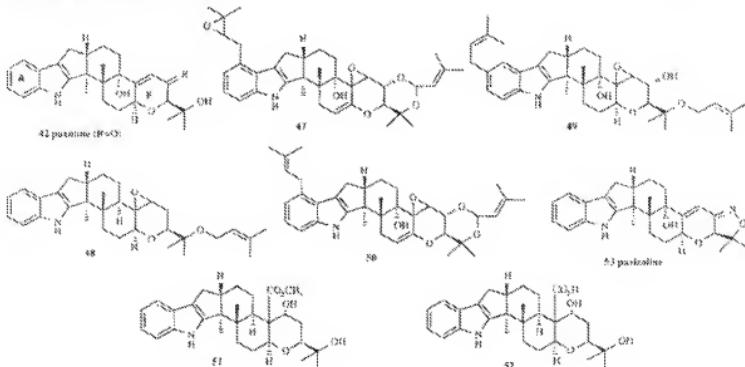
μM , 39 and 40 caused small decreases in channel open probability, whereas 41 had no clear effect.

Despite the structural dissimilarity of the three terpenoid series described above (saponins, monoketol and L-735,334), the active components of all three display similar biological profiles. All of them displace labeled CnTX in a concentration-dependent manner, fail to fully displace CnTX, and activate BK channels in electrophysiology experiments when applied to the cytoplasmic side of the channel. These data are consistent with the notion that the compounds do not act near the toxin binding site, but are allosteric modulators of toxin binding [92].

BK Channel Blockers

As discussed above, BK channels are very effectively blocked by the wasp peptide toxin charybdotoxin (CnTX) [93/94] and the related peptide iberiotoxin (IbTX) [41]. These peptides have been instrumental in the biophysical characterization of BK

Table 7. Indole Diterpen Alkaloid BK Blockers



Compound	50 % BK Channel Block (nM)
42 (pascoline, R=O)	1
43 (pascoline, R=OH, H, H)	100,000
44 (pascoline, R=OH, H)	100,000
45 (pascoline, R=OH, H)	NA ^a
46 (pascoline, R=OH)	NA ^a
47	1
48	<1
49	160
50	100
51	1500
52	>10,000
53 (pascoline)	10 ^b

^a No binding observed at concentrations up to 100 μM

^b No test for binding > 100 μM

channels as well as the development of BK modulator pharmacology. However, their potential therapeutic utility may be limited due to the inherent disadvantages associated with the synthesis, delivery, metabolism and tissue penetration of peptides. Therefore, in the context of this review, the peptide toxins will be considered only as useful tools for the study of BK channels.

Terpenoids

Using the ^{125}I -CnTX screen described in the terpenoid BK opener section, the Merck group has also reported a series of indole diterpene alkaloids, some of which inhibited binding and some of which enhanced toxin binding. Regardless of their effects on binding, both types of compounds demonstrated an ability to block BK channels in excised inside-out patches from bovine aortic smooth muscle cells [95,96]. In contrast to the BK-opening alkaloids, which only act from the cytoplasmic side of the membrane, the indole alkaloids block BK channels when applied to either the inner or outer surface of the channel. As can be seen in Table 7, alkaloid 47 is extremely potent, blocking 92% of BK channels at a concentration of 0.1 nM. Removal of the sidechain epoxide of 47 to give 50 decreased activity. Paxilline (42) is a potent compound, blocking greater than 50% of the BK channels at a concentration of 10 nM. One recurring theme among the indole terpenes is the detrimental effect upon introduction of a polar substituent on the F ring. Reduction of the ketone of paxilline to give either α -D (43) or β -D (44) reduced BK activity (50% channel block \approx 100-1000 nM). Replacement of the ketone with an amine (*p*-prolylamine, 45) or ketoxime (*p*-oxilline ketoxime, 46) resulted in analogs which were devoid of activity in binding experiments. Introduction of alcohols onto the E and F rings of the core of epoxide 48 in gave 49 decreased the channel blocking activity by raising the 50% channel blocking concentration from less than 1 μ M to 100 nM. Allylation of the F-ring alcohol of 49 by forming the cyclic ketal 50 results in an analog of intermediate activity. Similarly, the activity of hydroxy ester 51, which is weakly active as a channel blocker, is reduced even further upon hydrolysis of the ester to carboxylic acid 52. The binding activity of paxilline (53), the cyclized paxilline, is biphasic; at low concentrations, it stimulated CnTX binding, while partial inhibition of CnTX was observed at higher concentrations. However, when evaluated for channel-blocking properties, paxilline induced a 50% decrease in channel activity at 10 nM.

Similarly to the BK-opening alkaloids, binding experiments support the idea that the terpenoid blockers interact at distinctly different locations from the toxin site of binding [95]. This concept has been extended further, when paxilline was applied in different concentrations to oocytes expressing *hSlc*, it was observed that, unlike the peptide blockers RTX and CnTX, the paxilline inhibition of *hSlc* current was characterized by at least two components. The resulting concentration-response relationship was best fit assuming 2 sites of interaction: a high affinity (9.1 nM) and a low affinity site (0.55 μ M) [64].

The alkaloid *terrandine* (Fig. 8, 54) has been used clinically in China for centuries in the treatment of a variety of diseases, including hypertension, cardiac arrhythmia and angina pectoris [97]. Subsequently, it has been shown to interact with voltage-activated L type and T type calcium channels and calcium-activated potassium channels. There are conflicting results regarding the BK channel blocking activity of terrandine; it was initially shown to induce a blocky block of BK channels from isolated terminal of the rat mesenteric pith [20], however, more recent experiments ob-

serve BK channels reconstituted from rat fast-twitch muscle microsomes [97] and from oocytes expressing *hSlc* [64] have failed to demonstrate any effect with terrandine.

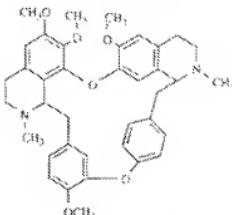


Fig. (8). The BK channel blocker terrandine

Small Molecule BK Blockers

Very few small molecule BK blockers have been described in the literature. Quaternary amines such as tetraethylammonium ion (TEA) block BK channels [98,99], but also block a variety of other ion channels [100,101] and are therefore of limited utility. The neuroleptic drug trifluoperazine (dihydrochloride) (Fig. (9), 55) was shown to block the open state of BK channels as measured in single channel recordings from excised inside-out patches obtained from dissociated rat hippocampal neurons [102]. It is speculated that

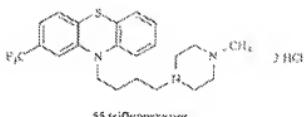
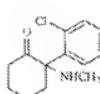
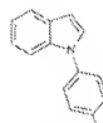


Fig. (9). Small molecule BK channel blockers



56 ketamine



57 DOI

trifluoperazine dihydrochloride ($pK_1 = 9$, $pK_2 = 8.1$) enters the cell in a neutral form from the extracellular space ($pI = 7.4$) and intracellularly ($pI = 7.0$) becomes charged, whereupon it enters the internal pore and blocks the BK channel. Trifluoperazine has also been reported to block sodium and calcium currents, consistent with a non-channel-specific mechanism of blockade [103]. The imidazole anesthetic ketamine (56) has also been reported to block BK channels when measured using cell-attached and excised patch single channel as well as whole cell recordings from GH3 cells [104]. However, similar to the lack of activity of imidazole *desmethyl* (57), ketamine did not produce a significant block of BK channels in oocytes expressing *hSlc*, even at the high concentrations (10 μ M) [64]. Oocyte made out patches from two sonic

most smooth muscle cells, indole 57 was shown to block BK channels at a concentration of $20 \mu\text{M}$ [104]. Indole 57 also blocked channels obtained from porcine tracheal Ca^{2+} cells. Because structural similarity exists between indole 57 and benzimidazolone NS (634), one might speculate that these compounds are acting at the same site, or stabilizing different open and closed channel states. Further studies will be needed to determine if a common site of action for both agonists and blockers can be identified.

Potential Therapeutic Utility of BK Modulators

BK channels are expressed in mammals in a wide variety of tissues and cells, including neurons [105], pancreas [106], skeletal muscle [107] and mammalian smooth muscle such as trachea [108], colon [109], and bladder [47,109,110]. They are also found within the vasculature, including coronary [68] and cerebral vessels [111]. Because of their widespread tissue distribution and dependence on both voltage and calcium for activation, they are seen as a potential therapeutic target for numerous disorders. Openers offer the opportunity to intervene in the consequences of neuronal and muscular hyperexcitability, while blockers may be particularly useful in the enhancement of synaptic transmission and the restoration of cognitive function in some neurodegenerative disorders. Compared to the number of studies which examined BK channel modulators electrophysiologically at the cellular or single channel level, there have been substantially fewer studies which have looked at the effects of BK channel modulators in tissue preparations or in whole animals. In particular, there have been a paucity of studies carefully examining the effects of BK modulators in specific animal models predictive of activity in human disease. Currently, no therapeutic agent is known to have BK channel modulation as its mechanism of action, although this does not preclude discovery of agents of unknown mechanism that may, in fact, be BK channel modulators. Recently, several preliminary studies have begun to confirm the potential utility of these compounds.

BK Channel Openers

Anthonys

BK channels are thought to be the major channel K^+ channel subtype in airway smooth muscle [108], and therefore compounds which regulate the function of BK channels may have therapeutic potential for use as bronchodilators in the treatment of asthma. The peptide BK channel blockers ChTX [112-114] and hTx [115] have both been shown to be potent contractile agents on airway smooth muscle. β -adb compounds have also demonstrated an ability to modify the tracheal relaxant effects of β -adrenoceptor agonists. Using guinea pig trachea first contracted with carbachol and then treated with the β -adrenoceptor agonist and tracheal relaxant isoproterenol, either with or without the addition of hTx, the maximal relaxation achieved with isoproterenol was reduced in the presence of 180 nM hTx [115]. These findings indicate the BK channels may be coupled to β -adrenoceptors in airway smooth muscle and are further evidence that regulation of basal BK channels may offer an opportunity in the treatment of asthma.

Uterine Impingement and Cystic Hypermotility

In a model which is thought to predict the Lennard-Jones potential, the relaxing effects on postmenstrual and menstrual h are 38%.

administration of 3.46 μ M NS-1603 relaxed acetylcholine-contraction guinea pig lung in a dose-dependent manner [73]. NS-094 has also been reported to activate BK channels present in human and guinea pig bladder smooth muscle [110]. These results offer the possibility that BK channel openers may be beneficial in the treatment of conditions such as urge incontinence and gastric hypermotility by virtue of their ability to relax abnormally contracting smooth muscle.

Hyperension

BK channels openers may also serve as vasodilating agents and therefore be of use in the treatment of hypertension. In rat cerebral arteries, NS 1619 induced a concentration-dependent relaxation response of arteries contacted with histamine/H₁, although it was also found to block calcium currents in the same tissue, which may contribute to the relaxant effects [111]. Similarly, the relaxation of norepinephrine-stimulated guinea pig artery with the anti-hypertensive agents clonidine and hydrochlorothiazide, which are known calcium channel blockers, can be reversed upon the administration of BTK [117]. In one study, administration of 3-10 mg/kg of NS 604 and NS 1619 to normotensive or spontaneously hypertensive (SHR) rats by an undiluted route of administration did not reduce blood pressure [118]. However another study reported that intravenous administration of 20 mg/kg of NS 604 reduced mean arterial blood pressure in SHR rats by 15% [119]. In the latter study, no effect on blood pressure was observed at the 10 mg/kg dose and there was no effect on heart rate at either dose. NS 604 was also studied for cardiovascular effects in rats and found to be cardio-selective and cause a concentration-dependent reduction in ventricular pressure. However, these effects were not reversed after administration of BTK [68]. In this study, it was concluded that the cardiovascular effects of NS 604 could be ascribed to the blockade of calcium channels, rather than opening of BK channels. In rat cerebral arteries, BTK blocked BK channels, thereby depolarizing and constricting pressurized arteries with myo-epicardial tone [120]. Evidence was presented which suggests that the degree of myogenic tone is regulated in part, by activation of BK channels, and that these channels may serve as a negative feedback pathway to control the degree of membrane depolarization and vasoconstriction. The cardiovascular utility of BK channel modulators remains to be fully explored, particularly with compounds which do not effect other ion channels such as calcium channels.

Psychoses 1-6

The anti-psychotic activity of NS-1602 has been studied in the cocaine hyperactivity test [33]. Intraperitoneal administration of 10.30 mg/kg of NS-1602 before cocaine administration antagonized the cocaine-induced hyperactivity, presumably due to an inhibition of dopamine uptake.

Conclusions

MS-904 has been evaluated in a pentylentetetraole (PTZ)-induced seizure model in mice [78]. Intraperitoneal administration of 36 and 40 mg/kg significantly increased the amount of PTZ required to induce both tonic and tonic-clonic seizures. Administration of 40 mg/kg of MS-904 also increased the threshold of PTZ-induced lethality. These results suggest that SR₂ agonists may be useful in the treatment of strong seizure disorders, although their effects in other seizure models are less clear.

Stroke and Traumatic Brain Injury

The ability of NS 004 to reduce cerebral damage following stroke has been reported in two animal models of stroke [119]. In a gerbil model of transient global ischemia, when NS 004 was given at 60 mg/kg i.p., it significantly reduced hippocampal damage. In a permanent model of focal ischemia in the SHR rat, administration of NS 004 at 1, 5 or 10 mg/kg, i.v., reduced infarct damage when given 1 hour after occlusion of the middle cerebral artery. The 5 mg/kg dose was also shown to be effective when administered 2 hours post-occlusion. These studies are an indication that BK openers may provide a method of neuroprotection following stroke at doses which do not produce cardiovascular side effects (*vide supra*). Because many of the processes of neurodegeneration following traumatic brain injury and stroke are thought to occur by similar mechanisms, compounds which are neuroprotective in stroke are also being pursued as therapy for delayed or secondary neuronal damage following traumatic brain injury [121].

BK Channel Blockers

In comparison to the BK channel openers, even less is known about the potential therapeutic utility of BK channel blockers.

Depression

The BK channel blocker phenylindole 57 has been studied in model of despair as measured by a lack of mobility of mice after being suspended by their tails. When 57 was given i.p. at doses between 10 and 100 mg/kg, a dose-dependent reduction in immobility was observed [66]. It is noted in this patent that both antidepressant and psychostimulating drugs decrease immobility in this test.

Memory Impairment

The same patent which demonstrated the anti-depressant activity of indole 57 also provides data on the memory enhancing properties of this compound [66]. In a model which evaluates memory processes involved in social recognition, rats given an intraperitoneal dose of 57 at 7.5-30 mg/kg demonstrated a significant decrease in investigatory behavior, indicating memory enhancement.

Conclusion

A great deal of information has been gained with respect to BK channels and their modulators in a relatively short period of time. Studies on the first cloned channel from *Drosophila* appeared in 1991 [9] and the first small molecular weight openers were reported in 1992 [8]. Since that time, the mouse and human *Slc* channels have been cloned and expressed, a β subunit has been identified and its effects characterized, and a variety of openers and blockers of BK channels have been disclosed. Among the BK modulators, some rudimentary structure activity relationships are starting to emerge. Within the small molecules such as the benzimidazolones, benzopyrans and furanates, these data indicate that electron-deficient aryl systems with either a carboxylic acid or a suitable isoster may function as BK openers. In addition to the small molecules, several classes of natural products have been identified as both openers and blockers of BK channels. As interest in BK channel channels increase, undoubtedly, additional modulators will

be identified from both known and novel chemical entities. This endeavor will provide researchers with ever increasingly refined tools to further define the function and utility of BK channels.

Because BK channels occur in a diverse array of tissues, modulators of these channels could potentially have a profound impact on controlling diseases associated with BK channels. The discovery of numerous alternative *Slc* splice variants, and β subunit that can complex with the BK channel and confer differential activation, holds promise with respect to being able to target specific tissues with BK channel modulators. Although large advances have been made in the last few years, there still remains much to be learned with respect to BK channels. Little is known about the tertiary structure of the channels, either with or without subunit association, the site(s) of interaction of small molecular weight modulators, as well as the effect(s) of BK channels under normal and pathological conditions. The discovery of new BK channel-specific modulators and their evaluation in models of different diseases will determine the degree to which this class of channels represents a realistic therapeutic target.

References

- Hille, B. *Book: Channels of Excitable Membranes*. 2nd Edition. Redwood City, CA: Sinauer Associates, MA, 1994.
- Hodsi, T.; Zagotta, N. *W. N. Current opinion in neurobiology* 1993, 3, 283-290.
- Arnaud, C.; Mountjoy, A.; Grubb, P.; Ashfield, R.; Peeks, P.; Smith, P. A.; Sakimura, H.; Cotes, B.; Ashcroft, J. H.; Ashcroft, F. M. *Nature* 1996, 379, 540-548.
- Wen, S. W.; Weston, A. B. *Br. J. Pharmacol.* 1985, 88, 121.
- Bonfield, J. R.; Russell, K.; Tissot, D. *Pharmaceutical News* 1995, 2, 23-27.
- Pineau, J.; Batera, J. *Cors. Pharm. Design* 1995, 1, 391-406.
- McNamee, O. B.; Harris, G. H.; Giangreco, K. M.; Ferguson, P.; Reshen, J. P.; Addy, M. B.; Burke, J. P.; Kaczmarek, G. J.; Garcia, M. L. *Biotechnology* 1993, 11, 6138-6133.
- Olesen, S. P.; Wagen, P. *European Patent Application 0677819* A2, 1992.
- Akamatsu, N. S.; Robertson, G. A.; Ganetzky, B. *Science (Wash.)* 1991, 253, 551-555.
- Adelman, J. P.; Shen, H.-Z.; Kavanagh, M. P.; Warren, R. A.; Wu, Y. N.; Logothetis, A.; Bond, C. T.; North, R. A. *Neuron* 1992, 9, 209-216.
- Burke, J. P.; Tyndall, S.; Mc Cobb, D. P.; Wei, A.; Salmond, L. *Science (Wash.)* 1993, 261, 221-224.
- Duszak, S. L.; Tuszynski, J. T.; Ghochikyan, V. K. *Mol. Brain Res.* 1994, 27, 189-193.
- Pallackson, L.; Ganetzky, B. *Human Mol. Genetics* 1994, 3, 1239-1243.
- Tseng-Chang, J.; Pester, C. D.; Krause, J. O.; Metz, R.; Gadomski, N.; D'Champt, T. J.; Reungis, P. H. *Neuron* 1994, 11, 1315-1330.
- McCobb, D. P.; Powis, S. L.; Featherstone, T.; Long, C. L.; Sato, M.; Krause, J. O.; Salmond, L. *Am. J. Physiol.* 1995, 269, 1767-1777.
- Kumar, R. G.; Eberhart, A.; Grossmann, B.; Munro, P.; Kaczmarek, G. J.; Garcia, M. L. *Cell Signaling* 1993, 5, 363-376.

[17] Reinhart, P. H.; Chung, S.; Levine, J. B. *Neuron* 1989, 2, 1031-1041.

[18] Reinhart, P. H.; Chung, S.; Martin, B. L.; Bratton, D. L.; Levine, J. B. *J. Neurosci* 1991, 11, 1623-1639.

[19] Reinhart, P. H.; Levine, J. B. *J. Neurosci* 1995, 15, 4572-4579.

[20] Wang, G.; Lemus, J. R. *Pflügers Arch* 1992, 421, 558-565.

[21] McNamara, O. B.; Helms, L. M.; Paltajack, L.; Ganetzky, B.; Swanson, P.; Loudon, R. J. *Neuron* 1995, 14, 643-650.

[22] McNamara, O. B. *J. Bioenerg. Biomembr.* 1991, 23, 537-560.

[23] Moghly, K. L.; Paltajack, B. S. *J. Physiol. (Lond.)* 1983, 344, 584-604.

[24] Laforte, R.; Oberhauser, A.; Lubarska, P.; Alvarez, O. *Annu. Rev. Physiol.* 1989, 51, 385-399.

[25] Beuckfeldt, K.; Rojer, J.; Jackson, M. B. *J. Physiol. (Lond.)* 1992, 458, 41-67.

[26] Barritt, I. H.; Magleby, K. L.; Paltajack, B. S. *J. Physiol. (Lond.)* 1983, 331, 211-230.

[27] Bennett, C. J.; Bolton, T. B.; Lang, R. J.; Takewaki, T. *J. Physiol. (Lond.)* 1986, 371, 45-67.

[28] Padley, J.; Ruffy, B. *Biophys. J.* 1988, 53, 919-934.

[29] Hudspeth, A. J.; Lewis, R. S. *J. Physiol. (Lond.)* 1989, 400, 237-274.

[30] Moczydlowski, E.; Llinás, R. *J. Gen. Physiol.* 1983, 82, 511-542.

[31] Solaro, C. R.; Praktyka, M.; Doug, I. P.; Lang, C. J. *J. Neurosci* 1995, 15, 6110-6123.

[32] Wang, G.; Thorn, P.; Lemus, J. R. *J. Physiol. (Lond.)* 1992, 457, 47-74.

[33] Beuckfeldt, K.; Jackson, M. B. *J. Physiol. (Lond.)* 1994, 475, 2, 241-254.

[34] Chung, S.; Reinhart, P. H.; Martin, B. L.; Bratton, D. L.; Levine, J. B. *Science (Wash.)* 1991, 253, 560-562.

[35] Isha, N. P.; Hudspeth, A. J. *Proc. Natl. Acad. Sci. USA* 1994, 91, 7575-7582.

[36] Rohrbaugh, R.; Charlton, M. P. *J. Neurosci* 1992, 12, 297-303.

[37] Rohrbaugh, R.; Garcia, M. L.; Kaczmarek, G. J.; Charlton, M. P. *Neuron* 1993, 11, 645-655.

[38] Roberts, W. M.; Jacobs, R. A.; Hudspeth, A. J. *J. Neurosci* 1990, 10, 3664-3684.

[39] Wei, A.; Saloff, L. *Science (Wash.)* 1986, 233, 780-782.

[40] Canica, S.; Garcia, M. L.; Laemmli, R. *Biophys. J.* 1992, 63, 583-590.

[41] Gómez, A.; Giménez-Correig, C.; Reuben, I. P.; Roy-Campanella, L.; Feijen-Bouwman, P.; Kaczmarek, G. J.; Garcia, M. L. *J. Biol. Chem.* 1990, 265, 11083-11090.

[42] Ganetzky, B. M.; Garcia, M. L.; McNamara, O. B. *Biochemistry* 1992, 31, 6149-6157.

[43] Miller, C. *Neuron* 1995, 15, 5-10.

[44] Sapp, E. B.; Garcia, M. L.; Reshen, J. P.; Panchett, A. A.; Kaczmarek, G. J. *J. Biol. Chem.* 1990, 265, 18745-18748.

[45] Asano, M.; Matsuzawa-Ito, K.; Matsuda, T. *Br. J. Pharmacol.* 1993, 108, 214-227.

[46] Asano, M.; Matsuzawa-Ito, K.; Matsuda, T.; Suzuki, Y.; Oyama, H.; Shirota, M.; Suga, Y. *J. Auton. Nerv. Syst.* 1994, 49, 0165-1838.

[47] Suzet-Kurtz, G.; Garcia, M. L.; Kaczmarek, G. J. *J. Pharmacol. Exp. Ther.* 1991, 259, 438-443.

[48] Beuckfeldt, K.; Jackson, M. J. *Neuropharmacol.* 1993, 20, 284-298.

[49] Carl, A.; Bowen, S.; Colbran, C. H.; Sanders, K. M.; Hume, J. R. *Pflügers Arch.* 1992, 411, 67-76.

[50] Denton, D. D.; Duchaine, P.; Eaton, D. C. *Brain Res.* 1994, 658, 61-68.

[51] Knauf, H.-G.; Schwaege, C.; Koch, R. O. A.; Eberhart, A.; Kaczmarek, G. J.; Glassman, H.; Wunder, F.; Pouys, O.; Garcia, M. L.; Sperk, G. *J. Neurosci.* 1996, 16, 955-963.

[52] Laike, R. *Molecular workings of large conductance (maxi) Ca^{2+} -activated K^{+} channels*, Laike, R., Ed.; Academic Press: San Diego, 1994, pp 79-104.

[53] Sellers, A. J.; Ashton, M. L. *Br. J. Pharmacol.* 1994, 111, 659-661.

[54] Papazian, D. M.; Schwarz, T. L.; Tempel, B. L.; Jan, Y. N.; Jan, L. Y. *Science (Wash.)* 1987, 237, 749-753.

[55] Elkins, T.; Gonçalves, B.; Wu, C.-P. *Proc. Natl. Acad. Sci. USA* 1988, 83, 8415-8419.

[56] Shen, K. Z.; Laike, R.; Davies, H. W.; Standen, N. B.; Adelman, J. P.; North, R. A. *Pflügers Arch.* 1994, 426, 449-445.

[57] Wei, A.; Saloff, C.; Lingle, C.; Saloff, L. *Neuron* 1994, 12, 671-681.

[58] Knauf, H.-G.; Folandet, K.; Garcia-Calvo, M.; Garcia, M. L.; Kaczmarek, G. J.; Sants, M.; Swanson, R. *J. Biol. Chem.* 1994, 269, 17274-17278.

[59] Swerdfeger, S. L.; Bolis, J. C. G.; Lum-Ragan, J. T.; McKay, M. C.; Post-Munson, D. J.; Trojnacki, I. T.; Chang, C.-P.; Grubhoff, V. K. *J. Neurosci.* 1996, 16, 4543-4550.

[60] Olsen, S.-P.; Jensen, I. H.; Meldt, P. *EP 017023 A2*, 1994.

[61] Olsen, S.-P.; Munch, B.; Wanjen, F.; Drejer, J. *NeuroReport* 1994, 5, 1001-1004.

[62] Olsen, S.-P.; Wanjen, F.; Hayes, A. G. *Br. J. Pharmacol.* 1993, 110, 29-39.

[63] Macmillan, S.; Sheridan, R. D.; Chilvers, E. R.; Parmod, L. Sr. *J. Pharmacol.* 1995, 110, 1656-1660.

[64] Grubhoff, V. K.; Lum-Ragan, J. T.; Beaufort, C. G.; Post-Munson, D. J.; Mewaroli, N. M.; Sturte, J. E. Jr.; Kozlowski, E. S.; Trojnacki, I. T.; Swerdfeger, S. L. *Mol. Pharmacol.* 1996, 50, 206-217.

[65] Wang, L.; Xu, X.; Lee, R. S.; Wang, J.; Xu, X.; Lee, K. S., Ed.; PAPER 1, Anaheim, CA, 1994, Vol. 8, pp A369 Poster 3295.

[66] Olsen, S.-P.; Munch, B.; Meldt, P.; Drejer, J. *Br. J. Pharmacol.* 1994, 112, 53-59.

[67] Edwards, G.; Niederer, Billeberg, A.; Schneider, J.; Nuack, T.; Weston, A. H. *Br. J. Pharmacol.* 1994, 113, 1518-1542.

[68] Sargent, C. A.; Grover, G. L.; Antonacopoulou, M. J.; McCullough, J. X. *J. Pharmacol. Exp. Ther.* 1993, 266, 1422-1429.

[69] McKay, C. M.; Dworczyk, S. J.; Maxwell, N. A.; Olsen, S.-P.; Reinhart, P. H.; Levine, J. B.; Adelman, J. P.; Grubhoff, V. K. *Neurophysiol.* 1994, 71, 1873-1882.

[70] Chomppay, G.; Inaki, J. L.; Puchelli, E.; Dalemans, W.; Grubhoff, V. K.; Himszky, J.; Dotti, G.; Barby, F.; Poirier, A.; Lachamki, M. *EMBO J.* 1995, 14, 3417-3421.

[71] Grubhoff, V. K.; Chomppay, G.; Barby, F.; Dworczyk, S. J.; Maxwell, N. A.; Lachamki, M. *J. Biol. Chem.* 1996, 269, 16181-16186.

[72] McAneney, N. A.; Si, S. Y.; Gao, J.; Boujard, C. G.; Lum-Ragan, J.; Gibbons, V. K. *Biorg. Med. Chem. Lett.* 1996, 6, 1641-1646.

[73] Olsen, S. P.; Moldt, P.; Pefanisou, O. World Patent Application WO 94/22837, 1994.

[74] Hu, S. H.-S.; K. Eink, C. A. *Eur. J. Pharmacol.* 1995, 294, 357-360.

[75] White, M. M.; Aylwin, M. *Mol. Pharmacol.* 1990, 37, 720-724.

[76] Chao, A. C.; Morizuka *Life Sciences* 1992, 51, 1453-1457.

[77] Otilio, M.; Tute, L. *Biophys. J.* 1994, 67, 2772-2779.

[78] Xu, X.; Tsai, T. D.; Wang, J.; Lee, E. W.; Lee, K. S. *J. Pharmacol. Exp. Ther.* 1994, 271, 362-369.

[79] Lee, K.; Rowe, L. C. M.; Axford, M. L. J. *Eur. J. Pharmacol.* 1995, 280, 215-219.

[80] Miyoshi, H.; Nakaya, Y.; Sato, K.; Kishii, F.; Takakura, M.; Nomura, M. *Life Sciences* 1995, 56, 291-298.

[81] Koh, D. S.; Reid, G.; Vogel, W. *Neurosci Lett* 1994, 165, 167-170.

[82] Geinbaud, C. H.; Loosje, N. J.; Van Breenen, C. *Eur. J. Pharmacol.* 1989, 167, 201-210.

[83] Balwierzak, J. L.; Krutin, C. M.; Kim, H. S.; Delgrande, B.; Worts, G. B.; Hu, S. L. *Naunyn-Schmied Arch. Pharmacol.* 1995, 352, 213-221.

[84] Ellory, J. C.; Culliford, S. J.; Smith, P. A.; Walowick, M. W.; Kausz, E. B. *Br. J. Pharmacol.* 1994, 111, 903-905.

[85] Pagan, L.; Rossi, J. I.; Bockaert, J. *Pflügers Arch.* 1994, 429, 176-182.

[86] Ulbachs, K.; Herne, H. G.; Junge, B.; Schöbe-Loos, R.; Sonnenrein, H.; Winkla, R.; De Vry, J. M. V. German Patent Appl DE 442678-A1, 1996.

[87] Koho, L.; Kuro-Hori, I.; Ishiguro, K.; Chowdhury, S. K.; Sanchez, Y.; Ogura, T. *Biorg. Med. Chem. Letters* 1994, 4, 1443-1446.

[88] Dhananj, V.; Vijayam, M. *Acta Cryst.* 1988, 44A, 409-412.

[89] Singh, B. S.; Goetz, M. A.; Zink, D. L.; Domrowski, A. W.; Pashook, J. D.; Gaudia, M. L.; Schmalhofer, W.; McManus, O. B.; Kaczorowski, G. J. *J. Chem. Soc. Perkin Trans I* 1994, 3349-3352.

[90] Garcia, M. L.; Giacobbe, R. A.; Hamers, O. D.; Kaczorowski, G. J.; Lee, S. H.; McManus, O. B.; Zink, D. L. International Patent Application WO 95/16442, 1995.

[91] Lee, S. H.; Hamers, O. D.; Helms, G. L.; Lisch, J. M.; Zink, D. L.; Giacobbe, R. A.; Bilek, G. P.; Stevens-Milne, S.; Garcia, M. L.; Schmalhofer, W. A.; McManus, O.; Kaczorowski, G. J. *Nature Prod.* 1995, 58, 1825-1828.

[92] Giacobbe, R. A.; Bilek, G. P.; Garcia, M. L.; Domrowski, A. W.; Sabine, G.; Kaczorowski, G. J.; Zink, D. L.; Bilek, G. P.; Goetz, M. A.; Schmalhofer, W. A.; Singh, S. B. *Biorg. Med. Chem. Lett.* 1995, 5, 731-734.

[93] MacKinnon, R.; Miller, C. *J. Gen. Physiol.* 1988, 91, 335-349.

[94] Anderson, C. S.; MacKinnon, R.; Smith, C.; Miller, C. *J. Gen. Physiol.* 1988, 91, 317-331.

[95] Kausz, H.-G.; McManus, O. B.; Lee, S. H.; Schmalhofer, W. A.; Garcia-Castro, M.; Helms, L. M. B.; Sanchez, M.; Giangrande, R.; Reuban, J. P.; Smith, R. A. B.; Kaczorowski, G. J.; Garcia, M. L. *Biochemistry* 1994, 33, 5819-5826.

[96] Garcia, M. L.; Giacobbe, R. A.; Hamers, O. D.; Lee, S. H.; McManus, O. B. International Patent Application WO 95/16771, 1995.

[97] Wang, G.; Lerner, J. R. *Life Sciences* 1995, 56, 295-306.

[98] Villarrubia, A.; Alvarez, O.; Obregon, A.; Latres, R. *Pflügers Arch.* 1998, 437, 118-126.

[99] Blaz, A.; Magleby, K. *J. Gen. Physiol.* 1984, 84, 1-23.

[100] Hermann, A.; Cormann, A. *J. Gen. Physiol.* 1981, 78, 87-110.

[101] Amatruda, C. *Physiol. Rev.* 1992, 72, 55-813.

[102] Beurrois, Y.; Yoshida, A.; Oda, M. *Eur. J. Pharmacol.* 1992, 216, 191-198.

[103] Ogata, N.; Toshin, M.; Neubashi, T. *J. Physiol. (Lond.)* 1990, 426, 165-183.

[104] Olsen, S. P.; Jensen, L. H.; Moldt, P.; Thamming, M. U.S. Patent 5158969, 1992.

[105] Meoch, R. W. *Annu. Rev. Biophys. Biogeo.* 1978, 7, 1-8.

[106] Findlay, L.; Dunne, M. J.; Peterkin, O. H. *J. Membr. Biol.* 1985, 69, 169-175.

[107] Lerche, H.; Fahke, C.; Iaizzo, P. A.; Lehmann-Hoyer, F. *Pflügers Arch.* 1995, 426, 738-757.

[108] McCann, J. D.; Welsh, M. J. *J. Physiol. (Lond.)* 1986, 372, 113-127.

[109] Trivedi, S.; Potter-Lee, L.; McConville, M.; Li, J. H.; Kao, S. T. *FASEB* 1994, 8, A59, 8529.

[110] Zagzagis, P.; Li, J. H.; Kao, S. T. *Pharmacology* 1992, 50, 13-18.

[111] Holland, M.; Langton, P. D.; Standen, M. B.; Boyce, J. P. *Br. J. Pharmacol.* 1996, 117, 119-129.

[112] Jones, T. R.; Charrette, L. *J. Gen. Physiol.* 1987, 90, 21-47.

[113] Jones, T. R.; Charrette, L.; Champion, E. *Eur. J. Pharmacol.* 1990, 182, 231-238.

[114] Jones, T. R.; Charrette, L.; Garcia, M. L.; Kaczorowski, J. *J. Pharmacol. Exp. Ther.* 1990, 255, 697-705.

[115] Jones, T. R.; Charrette, L.; Garcia, M. L.; Kaczorowski, G. J. *J. Appl. Physiol.* 1993, 74, 1879-1884.

[116] Trivedi, S.; Potter-Lee, L.; Li, J. H.; Yasay, G. D.; Russell, K.; Ohmacht, C. J.; Empfield, J. H.; Traub, D. A.; Kao, S. T. *Biophys. Biophys. Res. Commun.* 1995, 211, 404-409.

[117] Calfee, J. A.; Schachter, M.; Severt, P. S. *J. Pharmacol. Exp. Ther.* 1993, 265, 1173-1180.

[118] Olsen, S. P. *Eur. Opin. Invest. Drugs* 1994, 3, 1181-1188.

[119] Moon, S. J.; Lembardo, L.; Hibshani, I.; Myung, H.; Landell, D.; McManus, O. *Br. Neurosci. Abstr.* 1993, 674.9.

[120] Brayden, J. E.; Nelson, M. T. *Science (Wash. U.)* 1992, 256, 532-533.

[121] McManus, O. J. *Neurotransmitter* 1993, 10, 215-261.